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Effects of phosphorus and potassium addition on growth and nodulation of *Dalbergia sissoo* in the nursery

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Abstract: A study was conducted to test the influence of different inorganic fertilizers (phosphorous and potassium) on the nodulation and growth of *Dalbergia sissoo* grown in the nursery. Before seeds sowing, different combinations of P, K fertilizers were incorporated with the nutrient-deficient natural forest soils, and then amended with cowdung (soil: cowdung = 3:1). Nodulation status (nodule number, shape, fresh weight, dry weight and color) in the roots and the plant growth parameters (length of shoot and root, collar diameter, fresh and dry weight of shoot and root) were recorded 60 days after seeds sowing. Nodulation status and growth of the plants varied significantly (P<0.05) in the soils amended with fertilizers in comparison to the control. The highest nodule number (62), fresh (0.50 g) and dry (0.07 g) weights were recorded with the dose of PK at the rate of 160 kg/hm^2. Nodule shape and color also varied widely in different treatments. In case of plant growth parameters, shoot and root length, collar diameter, fresh and dry weight of the plants took on a significant difference (P<0.05) among various combination of fertilizers. From the study, it is revealed that PK at the rate of 160 kg/hm^2 fertilizer with soil and cowdung mixture (soil: cowdung = 3:1) is recommended for optimum growth and nodule formation of *D. sissoo* in degraded soils at a nursery level.

Keywords: *Dalbergia sissoo*; Inorganic fertilizers; Nodulation; Seedling growth; Nursery

Introduction

Dalbergia sissoo Roxb. is an important multipurpose tree species, belonging to leguminosae subfamily Papilionoideae, and widely used in agroforestry, afforestation programs and farm forestry in Indian subcontinent (Tewari 1994; Shayesta 2000). The species is easily planted and managed, and has a wide range of end uses (Sah *et al.* 1998). Due to its natural hardiness and fast growth rate, *D. sissoo* is used for furniture and general construction purposes (Troup 1921; Tewari 1994). This species is also extensively being planted in Bangladesh. It is estimated that about 60% of the plantations in north and south, and 20% in the central and eastern regions are planted with *D. sissoo* in Bangladesh (Baksha and Basak 2000). The plants are mainly used in community forests, private forests, woodlot plantations, or around the homesteads in many districts of the country (Webb and Hossain 2005).

Fertilizer has an important effect on plant nodulation, N_2 fixation and seedling growth. Nitrogen fixation should reduce or eliminate when the soil has high supplies of ammonium and nitrate (MacDicken 1994). Nitrogen-fixing trees are ideal for afforesting degraded soil because of their ability to establish and thrive in nitrogen deficient soils (MacDicken 1994; Rahman *et al.* 2004). If the fertility of degraded soil is to be maintained, it has to be replenished either by fresh supply of fertilizers or by plants that regularly fix atmospheric N_2. Although inorganic fertilizers are expensive to use over vast plantation areas, they greatly influence growth and formation of nodules (Pankaj *et al.* 1998).

Plants engaged in symbiotic N_2 fixation generally have a high requirement for P (Robson 1983; Jungk 1998), which was mainly contributed by high ATP requirements for nitrogenase function (Ribet and Drevon 1996; Al Niemi *et al.* 1997), plus P needs for signal transduction, membrane biosynthesis, and nodule development and function (Graham and Vance 2000). P has been shown to increase plant growth and stimulate nodulation in legumes (Gates 1974; Gates and Wilson 1974; Robson *et al.* 1981; Jakobsen 1985; Israel 1987; Hellsten and Huss-Danell 2000) as well as in other plants (Quispel 1958; Ekblad and Huss-Danell 1995; Yang 1995; Reddell *et al.* 1997). The effects of P on nodulation and nitrogenase activity were often ascribed to a general stimulation via plant growth (Robson *et al.* 1981; Jakobsen 1985; Yang 1995; Reddell *et al.* 1997). However, some investigations suggested that P had a specific stimulation on different plant nodulations (Israel 1987; Hellsten and Huss-Danell 2000).

Much research has been done on the effects of fertilizers on growth and nodulation in different legumes (Becker *et al.* 1991; Paulino *et al.* 1995; Ginwal *et al.* 1995; Perez *et al.* 1996; Verma *et al.* 1996; Datta and Das 1997; Reddell *et al.* 1997; Anegbeh and Tchoundjeu 2002; Gentili and Huss-Danell 2002). But less information is available in the case of *D. sissoo,* especially in soil conditions of Bangladesh. This study is an attempt to record the growth and nodulation status of *D. sissoo* in natural degraded soils amended with different inorganic fertilizers (P and K) at the

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nursery.

Materials and methods

Site selection and poly bag preparation

The study was conducted during five months from April to August in 2005 at the nursery of Institute of Forestry and Environmental Sciences, Chittagong University (IFESCU), Chittagong, Bangladesh. For potting media, natural degraded soils of Chittagong University campus and well decomposed cowdung were sieved (<3 mm) and mixed thoroughly in a ratio of 3:1. Cowdung was collected locally and deposited in the IFESCU nursery to decompose for about two months. 9″×6″ poly bags were used for the experiment. Triple Super Phosphate (P_2O_5, 48%) and muriate of potash (K, 50%) fertilizers with different combinations were applied to the soils. Seed sowing was done three days after mixing the fertilizers in the soil.

Seed collection and Experimental design

Pods were collected from the plus (healthy selected) *D. sissoo* trees of the Chittagong University campus. They were dried in the sun and then seeds were extracted. Uniform seeds were selected for the experiment. The seeds were sown in the polybags and allowed to grow up to 60 days.

There were altogether 330 seedlings equally divided into 11 treatments. A Complete Randomized Design (CRD) was adopted with three replications. Total 990 seeds were sown in eleven different treated soils. Three replications of each treatment comprised of ninety seeds. The seeds were sown at equal depth in each plot. The treatments used in the experiment were as follows:

Group	Treatment
T_0	Control (Only Soil)
T_1	Soil + Cowdung
T_2	Soil + Cowdung+ P @ 80kg/hm^2
T_3	Soil + Cowdung+ P @ 160kg/hm^2
T_4	Soil + Cowdung+ P @ 320 kg/hm^2
T_5	Soil + Cowdung+ K @ 80kg/hm^2
T_6	Soil + Cowdung+ K @ 160kg/hm^2
T_7	Soil + Cowdung+ K @ 320kg/hm^{2}v
T_8	Soil + Cowdung+ P K (P: K= 1: 1) @ 80 kg/hm^2
T_9	Soil + Cowdung+ P K (P: K= 1: 1) @ 160 kg/hm^2
T_{10}	Soil + Cowdung+ P K (P: K= 1: 1) @ 320 kg/hm^2

Assessment of growth and nodule parameters

The seedlings were harvested 60 days after seeds sowing. Nine seedlings from each treatment were randomly selected and carefully collected with the entire roots intact. Then physical parameters of the nodules (nodule number, fresh and dry weight, color and shape) and seedlings (shoot and root length, collar diameter, the total number of leaf and leaflets, fresh and dry shoot and root weight) were assessed. Collar diameter was measured by using slide calipers. After taking the data of above mentioned parameters, the seedlings were oven-dried at 70ºC for 48 h until the constant weight was obtained.

All the data were analyzed statistically by using the computer software package SPSS and were subjected to analysis by DMRT.

Results

Nodulation status of seedlings

The nodule number, fresh and dry weight, color and shape of *D. sissoo* in different treatments were shown in Table 1. The nodulation was found to greatly affect by the fertilizer combination of P and K. In all cases the nodule number was significantly (P<0.05) increased at T_9 (62) and followed by T_{10}, T_5 compared to T_0. The highest nodule fresh weight (0.50 g) was also recorded in T_9 followed by T_{10}, T_4, T_5, and the lowest (0.07 g) in the control. The maximum nodule dry weight was found in T_9 (0.07 g) while the minimum in T_0 (0.01 g). Most of the nodules were brown (T_2, T_3, T_4, T_5, T_6, T_8, T_{10}), some were whitish brown (T_0 and T_1), while only T_7 and T_9 showed whitish and pinkish color. The nodule shape of *D. sissoo* varied considerably from globose to oblate (T_6, T_7, T_8), globose to oblate & elongate (T_9, T_{10}), elongate & coralloid (T_4), globose & coralloid (T_1), globose & elongate (T_3) and globose (T_0, T_2, T_5). Moreover, all the nodules were distributed in the secondary roots.

Table 1. **Nodule number, fresh and dry weight, color and shape in different treatments of 60 days old *D. sissoo* seedlings**

Treatment	Nodule number	Nodule fresh weight (g)	Nodule dry weight (g)	Nodule color	Nodule shape
T_0	5.67 d*	0.07 e	0.01 c	Whitish brown	Globose
T_1	14.67 cd	0.13 cde	0.02 bc	Whitish brown	Globose, Coralloid
T_2	17 bcd	0.10 de	0.02 bc	Brown	Globose
T_3	30.33 bcd	0.16 cde	0.03 bc	Brown	Globose, Elongate
T_4	39 bc	0.37 b	0.05 ab	Brown	Elongate, Coralloid
T_5	40.67 abc	0.23 bcd	0.05 ab	Brown	Globose
T_6	16.33 bcd	0.13 cde	0.02 c	Brown	Globose to oblate
T_7	29.67 bcd	0.10 de	0.03 bc	Whitish	Globose to oblate
T_8	19.67 bcd	0.17 cde	0.01 c	Brown	Globose to oblate
T_9	62 a	0.50 a	0.07 a	Pinkish	Globose to oblate, Elongate
T_{10}	46 ab	0.37 b	0.06 a	Brown	Globose to oblate, Elongate

* Means followed by the same letter (s) in the same column do not vary significantly at P<0.05, according to Duncan's Multiple Range Test (DMRT).

Morphological growth parameters of seedlings

As shown in Table 2, shoot growth was significantly (P<0.05) different among all the treatments. The highest shoot growth was found in T_3 (41.83 cm) while the lowest was in the control (13.30 cm). The highest root length was recorded in T_9 (44.33 cm) and the lowest in T_5 (17.67 cm). The total root length was the longest in T_9 (81.66 cm) followed by T_3, which were significantly (P<0.05) longer than that of rest treatments. Maximum collar diameter was found in T_5 (3.98 mm) whereas the lowest in T_0 (2.17). Maximum leaf number (36.67) and maximum number of the leaflets (177.67) were both observed in T_9 as compared to the minimum number of the leaf (8.33) and the leaflets (30.33) both in T_0.

Table 2. Shoot and root length, collar diameter, number of leaves and leaflets in different treatments of 60 days old *D. sissoo* seedlings

Treatment	Length (cm)			Collar diameter	Number of leaves	Number of leaflets
	Shoot	Root	Total			
T_0	13.30 c*	22.83 ab	36.13 c	2.17 b	8.33 b	30.33 c
T_1	25.67 bc	29.17 ab	54.84 c	2.83 ab	21 ab	85.67 abc
T_2	32.87 ab	26.33 ab	59.20 ab	3.22 ab	16.67 ab	79 bc
T_3	41.83 a	33.67 ab	75.5 a	3.42 a	20.67 ab	92 abc
T_4	39.27 ab	29 ab	68.27 ab	3.65 a	17.33 ab	85 abc
T_5	34.30 ab	17.67 b	51.97 bc	3.98 a	24.33 ab	159.33 ab
T_6	37.67 ab	25.33 ab	62.97 ab	3.65 a	25.67 ab	118 abc
T_7	35.97 ab	33 ab	68.97 ab	3.33 a	16 ab	77.67 bc
T_8	36.17 ab	33 ab	69.17 ab	3.68 a	22 ab	105.33 abc
T_9	37.33 ab	44.33 a	81.66 a	3.80 a	36.67 a	177.67 a
T_{10}	36ab	31 ab	67 c	3.73 a	15.67 b	72 b c

* Means followed by the same letter (s) in the same column do not vary significantly at $P<0.05$, according to Duncan's Multiple Range Test (DMRT).

Fresh and dry matter production

Fresh and dry matter production, e.g. shoot, root and nodule fresh weight; total fresh weight; shoot, root and nodule dry weight and total dry weight were shown in Table 3. Shoot fresh weight was the highest in T_9 (11.03 g), which varied significantly ($P<0.05$) from other treatments. The lowest shoot fresh weight was recorded in T_0 (0.93 g). The maximum shoot dry weight was found in T_9 (3.30 g) and the minimum in T_0 (0.42 g). The highest root fresh weight was observed in T_4 (2.73 g) and the lowest in the control (1.16 g). The maximum root dry weight was recorded in T_5 (0.80 g) while the minimum in T_0 (0.52 g). However, both fresh and dry weight of root did not vary significantly ($P<0.05$). Nodule fresh weight was the highest in T_9 (0.50 g) and the lowest in T_0 (0.07 g). The maximum nodule dry weight was found in T_9 (0.07 g) and the minimum in T_0 (0.01 g). Both the total fresh (13.79 g) and dry (4.05 g) weight in T_9 were significantly ($P<0.05$) higher than those of rest treatments, and the lowest was found in T_0.

Table 3. Shoot fresh and dry weight, root fresh and dry weight, nodule fresh and dry weight in different treatments of 60-day-old seedlings of *D. sissoo*

Treatment	Fresh weight (g)				Dry weight (g)			
	Shoot	Root	Nodule	Total	Shoot	Root	Nodule	Total
T_0	0.93 d*	1.16 a	0.07 e	2.16 e	0.42 c	0.52 a	0.01 c	0.95 c
T_1	3.50 cd	2.40 a	0.13 cde	3.03 e	1.01 bc	0.79 a	0.02 bc	1.82 c
T_2	5.80 abcd	1.80 a	0.10 de	7.70 de	1.77 abc	0.58 a	0.02 bc	2.37 bc
T_3	6.40 abc	2.03 a	0.17 cde	8.60 cde	1.98 abc	0.45 a	0.03 bc	2.46 bc
T_4	7.90 abc	2.73 a	0.37 b	11 bc	2.54 ab	0.74 a	0.05 ab	3.33 bc
T_5	9.43 ab	2.46 a	0.23 bcd	12.09 b	2.82 a	0.80 a	0.05 ab	3.66 bc
T_6	7.46 abc	1.90 a	0.13 cde	9.49 bcd	2.34 ab	0.65 a	0.02 c	3.01 bc
T_7	5.40 bcd	1.47 a	0.10 de	6.97 e	1.64 abc	0.59 a	0.03 bc	2.26 bc
T_8	8.30 abc	2.10 a	0.17 cde	10.57 bcd	2.51 ab	0.70 a	0.01 c	3.22 ab
T_9	11.03 a	2.26 a	0.50 a	13.79 a	3.30 a	0.68 a	0.07 a	4.05 a
T_{10}	7.53 abc	2.17 a	0.37 b	10.07 bcd	2.33 ab	0.66 a	0.06 a	3.05 bc

* Means followed by the same letter (s) in the same column do not vary significantly at $P<0.05$, according to Duncan's Multiple Range Test (DMRT)

Discussion

The present study showed that nodulation status, morphological growth and fresh & dry matter production of *D. sissoo* seedlings were stimulated by the application of inorganic fertilizers. It was observed that the highest growth and nodule formation in *D. sissoo* was found in combination of phosphorous and potassium (P: K = 1:1) @ 160 kg/ha of soil amended with cowdung. These findings were in agreement with that of Becker *et al.* (1991) who revealed that nitrogen accumulation was higher with the application of P and K. The present study also supports the result of Abdel-Wahab (1985) who explained that roots were relatively richer in nitrogen (dry weight basis) in K-rich conditions. He also revealed that a significant amount of the nitrogen fixed was exuded at a low level of K supply. Paulino *et al.* (1995) examined the effect of K fertilization on growth, nodulation and mineral composition of *Leucaena leucocephala.* The present study is in agreement with the revelation of Gates (1974) and Israel (1993) that P deficiency appears to impact nodule function more directly. Reddell *et al.* (1997) also showed that P requirement for host plant growth was similar to, or higher than that required for symbiotic N_2 fixation processes.

The findings of the present study indicate that nodulation status (i.e. number of nodules, shape, color and fresh and dry weight of nodule) in *D. sissoo* recorded from different fertilizer treatments varied significantly. The data are compatible with the work of Datta and Das (1997) who reported a positive relationship between nodule dry weight and biomass. The findings also reveal that the use of inorganic fertilizers increase the nodule numbers and support the result of Razz *et al.* (1995) who examined the effect of inorganic fertilizers on nodulation status of plants. It was observed from the present study that increased fertilizer treatment resulted in the increase in growth and dry matter production of nodules, also supporting the result of nodulation status of *Samanea saman* investigated by Rahman *et al.* (2004). Gentili and Huss-Danell (2002) explained that P stimulated both nodule biomass and nodule number, and revealed the stimulation was specific for nodulation but not simply mediated via plant growth. It was found that the nodule shapes in *D. sissoo* were globose, elongated, coralloid, and oblate. Nodules were mostly brown in color and rough surfaced. Rests of the young nodules were smooth-surfaced while older nodules took on various shapes. These findings were consistent with the results of Anegbeh and Tchoundjeu (2002) who reported the characteristics of nodules of agroforestry tree species in Niger Delta, Nigeria.

The morphological parameters of the seedlings viz. length of shoot and root, collar diameter, number of leaves and leaflets were significantly stimulated with the application of inorganic fertilizers. The results of this study especially the total length and collar diameter are in agreement with that of Duguma *et al.* (1994) who explained height increment and stem diameter enlargement were good traits for selecting fast-growing leguminous plants. Rahman *et al.* (2004) explained that phosphorus increased root weight, but phosphorus alone had no effect on the number of nodules. Robson *et al.* (1981) and Jakobsen (1985) also recorded in their study that stimulation of N_2 fixation following P addition was through rapid enhancement of shoot growth, with a resultant influence on nodule parameters.

From the present study, it is found that the application of fer-

tilizers yielded better growth of D. sissoo seedlings. These findings are supported by Verma et al. (1996) who reported the positive effect of P fertilizers on the growth of D. sissoo.

Conclusion

Nitrogen-fixing trees are ideal for afforesting degraded soil because of their ability to establish and thrive in nitrogen deficient soils. The present study shows that the combination of phosphorous and potassium (P: K = 1:1) @ 160 kg/hm^2 of soil in addition to cowdung (Soil: cowdung = 3:1), if applied at nursery level, facilitates optimum growth and nodule formation in *D. sissoo*, and helps to obtain healthy seedlings that can be easily established in degraded sites.

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